CYCLIC LOAD INDUCED CALCIUM ACCRETION IN ROS CELLS.

A.J. Banes, G. Vadiakas, A. Keen, B. Brigman and M. Calderon, Dept. of Surgery, University of North Carolina, 253 Clin. Sci. 229H, Chapel Hill, NC 27514

An in vitro system was used to apply cyclic load to osteoblast-like cells in culture. Calcium accretion was quantitated in loaded or nonloaded cells with or without the calcium channel blocker, verapamil, to test the inhibitor's effects on calcium accretion. ROS 17/2.8 cells, were plated on flexible bottomed culture plates and subjected to a cyclic deformation regimen of 0.05 Hz, 0.24 maximum strain at 24% elongation for 3 and 7 days or continuously for up to 7 days. Group 1 (n=6) received load and 10 uM verapamil; group 2 was loaded without drug; group 3 had no drug or load. On days 3 and 7, replicate cultures were stained with alizarin red for calcium or labeled for the final 24 h with ^{45}Ca. Finally, PCR experiments were performed to indicate mRNA levels for alkaline phosphatase and osteopontin. Cyclic loading stimulated calcium incorporation 26% in ROS 17/2.8 cells at d7 but not d3. Alizarin red stained d7 cultures bright red, indicating calcium presence compared to the nonloaded control. Verapamil diminished load-induced calcium accretion without reducing accretion in control, nonloaded cells. The latter result with control, drug-treated cells may indicate that 10 uM verapamil affects channels other than calcium channels. In a daily drug addition experiment, verapamil reduced calcium accretion only if added prior to day 4. mRNA levels for AP and OP were increased by cyclic load. Cyclic loading may stimulate Ca channel activity, induce proteins involved in mineralization or alternatively, act at other receptors such as stretch-activated channels. supported by NIH AR38121.